REMARKS/ARGUMENTS

Status of the Claims

Upon entry of the present amendment, claims 1-11 and 17-19 are pending. Claims 1-6, 8-9 and 17 are amended to set forth a population of primary cultured adipocyte cells that are substantially free of non-adipocyte cells. Support is found, for example, in Figures 1, 4 and 6. New claims 17-18 are added. Support is found, for example, on pages 5, 11, and 21.

Objections to the Claims

The Examiner has objected to claims 1, 2, 5-7 and 9 because they have not been amended to recite the elected species. It is Applicants understanding that the requirement for electing a specific viral vector and specific gene was made as a *species election* requirement. Applicants understand that a species election requirement will be withdrawn upon a finding of allowability of claims generic to the species. *See*, M.P.E.P. § 809.02. Because Applicants believe that the presently amended claims are allowable over the cited art, no such amendment is required.

Rejection under 35 U.S.C. § 102(e)

The Examiner has rejected claims 1-9 and 17 under 35 U.S.C. § 102(e) as allegedly anticipated by U.S. Patent No. 7,015,037 ("Furcht"). To the extent that the present rejection applies to the amended claims, Applicants respectfully traverse.

The Examiner alleges that the rejected claims are "directed to an adipocyte, or a primary cultured adipocyte, or an implant composition comprising the same". The Examiner further alleges that "there is no evidence that adipocytes established from adipose tissue are structurally or functionally different from those established from differentiated mesenchymal stem cells", and maintains the rejection.

Applicants do not agree with the Examiner. However, in the interest of furthering prosecution, Applicants have amended claim 1 to set forth "a population of primary cultured adipocytes...wherein the population is substantially free of non-adipocyte cells".

Furcht discloses the isolation of bone marrow mononuclear cells (column 44, Example 1) and also discloses the differentiation of such cells (column 46, Example 2). However, Furcht does not describe the transduction of adipocytes that have been differentiated from adult stem cells. In Example 4, Furcht discloses that the EGFP gene was introduced into the bone marrow mononuclear cells with a retroviral vector and the cells were then induced to differentiate into osteoblasts, chondrocytes, stromal cells, skeletal and smooth muscle myoblasts and endothelial cells (column 48, lines 42-44). At column 49, lines 15-24, Furcht discloses that osteoprogenitor cells were confirmed to exist in marrow and these cells can differentiate into numerous cells types, including osteoblasts, chondrocytes, adipocytes, fibroblasts, and marrow stromal cells. However, Furcht does not show any data with respect to the purity of the obtained adipocytes.

As was discussed in the response to the previous non-final Office Action, bone marrow-derived stem cells of Furcht are multipotent stem cells that differentiate into various types of cells depending on culture conditions, and obtaining a homogeneous population of adipocytes from such bone marrow-derived stem cells is difficult. As evidence showing this point, Applicants attach hereto a reference by Jackson et al. (Journal of Postgraduate Medicine, 53(2):121-127, 2007).[1] Jackson et al. describe the cellular characteristics and differentiation potential of adult mesenchymal stem cells (MSCs), and they examined adipogenic potential of MSCs by a method similar to that utilized in Furcht (see column 46, lines 50-55 of Furcht and p. 123, left column of Jackson et al.). As a result, Jackson et al. confirmed the appearance of adipocytes containing lipid-filled droplets through staining with oil red O (Figure 1D). However, as is clear from the photograph shown as Figure 1D, the ratio of cells containing lipid-filled droplets (*i.e.*, adipocytes) in total cells was not high and the cell population obtained by the method of Jackson et al. clearly contains a substantial number of non-adipocyte cells.

In contrast, in the present invention, adipose tissues were extirpated under sterile conditions and washed, digested with collagenase and then separated into precipitate and suspended layer by centrifugation. The floating layer was washed and then subjected to ceiling

[1] Jackson *et al.*, *Journal of Postgraduate Medicine* (2007) 53(2):121-127 is attached as Exhibit A.

culture. After culturing, the cells adhering to the ceiling surface were collected by trypsin treatment and transferred to a normal culturing system. The cells were induced to differentiate into adipocytes by changing the medium to an induction medium comprising IBMX (3-isobutyl-1-methylxanthine), dexamethasone, and insulin and to maturation medium comprising insulin. *See*, Example 1 of the present application. After 14 days of ceiling culture, adhesion of adipocytes carrying lipid droplets was observed on the ceiling-side culture surface (Figure 1(A)). When these cells were transferred to a normal culturing system, they showed fibroblast-like growth, as shown in Figure 1(B). However, when differentiation was induced by IBMX, dexamethasone, and insulin, the cells again differentiated into mature adipocytes that carry lipid droplets (Figure 1(C)). Stored fat was stained red with oil red O staining (Figure 1(D)). As is clear from Figures 1(C) and 1(D), the cell population prepared by the method of Example 1 is much richer in adipocytes than cells obtained by Jackson et al., and is substantially free of non-adipocyte cells.

Since it is evident from the data shown in Jackson et al. that the adipocytes of Furcht would contain a substantial number of non-adipocyte cells, Furcht clearly does not anticipate the present invention. Accordingly, the Examiner is respectfully requested to withdraw the present rejection.

Rejections under 35 U.S.C. § 103(a)

Furcht in view of Crystal, Baetge

The Examiner has rejected claims 9-11 under 35 U.S.C. § 103(a) as allegedly rendered obvious over Furcht in view of Crystal, and further in view of Baetge. To the extent that the present rejection applies to the present claims, Applicants traverse.

As discussed above, the population of adipocytes obtained by the method of Furcht contains substantial numbers of non-adipocyte cells. Thus, the primary reference Furcht does not teach an implant composition comprising a population of primary cultured adipocytes which is substantially free of non-adipocyte cells. When using an implant comprising adipocytes for gene therapy, purity and homogeneity of adipocytes is very important in terms of safety.

While the method of Furcht cannot produce adipose cell population that is substantially free of non-adipocyte cells, the method of the present invention can. Furcht never teach a population of adipocytes which is substantially free of non-adipocyte cells. Moreover, they fail to even point out the importance of purity/homogeneity. The disclosures of Crystal and Baetge do not supply the elements missing from Furcht. Therefore, the presently claimed implant composition that is substantially free of non-adipocyte cells is not obvious from the combined disclosures of Furcht, Crystal and Baetge. This also holds true for the population of primary cultured adipocytes of claims 1-5, 17, and 18.

Furcht in view of Hertzel

The Examiner has rejected claims 6-7 are rejected under 35 U.S.C. § 103(a) as allegedly rendered obvious over Furcht in view of Hertzel. The Examiner concedes that Furcht does not teach the establishment of cultured adipocytes from adipose tissue. The Examiner alleges that the culture of primary adipocytes from adipose tissue for gene transfer was well known in the art, as disclosed by Hertzel, which discloses the *in vitro* adenoviral transfer of a reporter gene via an adenovirus vector to primary cultured murine adipocytes. The Examiner alleges that a person of ordinary skill in the art would have been motivated to combine the disclosures of Furcht and Hertzel, and to substitute primary cultured adipocytes for differentiated adipocytes as a matter of design choice, and to forego the isolation and differentiation of mesenchymal stem cells.

Applicants do not agree with the Examiner. However, in the interest of furthering prosecution, Applicants have amended claim 6 to set forth a method of producing a population of primary cultured adipocytes that are substantially free of non-adipcyte cells. The Examiner concedes that Furcht does not disclose the establishment of cultured adipocytes from adipose tissue. Furcht also does not disclose anything about preparing a population of adipocytes which is substantially free of non-adipocyte cells, and also fails to suggest the importance of purity/homogeneity of adipocytes when using an adipose cell population as an implant for gene therapy. These teachings are also clearly missing from Hertzel.

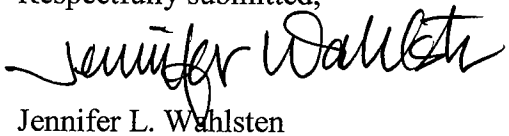
In view of the foregoing, the combined disclosures of Furcht and Hertzel do not render the present invention obvious. Accordingly, the Examiner is respectfully requested to withdraw this rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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Adult mesenchymal stem cells: Differentiation potential and therapeutic applications

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ABSTRACT

Adult mesenchymal stem cells (MSCs) are a population of multipotent cells found primarily in the bone marrow. They have long been known to be capable of osteogenic, adipogenic and chondrogenic differentiation and are currently the subject of a number of trials to assess their potential use in the clinic. Recently, the plasticity of these cells has come under close scrutiny as it has been suggested that they may have a differentiation potential beyond the mesenchymal lineage. Myogenic and in particular cardiomyogenic potential has been shown *in vitro*. MSCs have also been shown to have the ability to form neural cells both *in vitro* and *in vivo*, although the molecular mechanisms underlying these apparent transdifferentiation events are yet to be elucidated. We describe here the cellular characteristics and differentiation potential of MSCs, which represent a promising stem cell population for future applications in regenerative medicine.

KEY WORDS: Adipogenesis, chondrogenesis, mesenchymal stem cells, osteogenesis, regenerative medicine

'Adult stem cells' is the collective term used to describe postnatal stem cells which, as opposed to embryonic or fetal stem cells, persist throughout life and fulfill functions to repair or replace cells within certain tissues in response to traumatic events or natural cell turnover. Among tissues identified to harbor stem cells throughout postnatal life, bone marrow has been studied for many years as a source of both hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). Mesenchymal stem cells reside in the stromal fraction of the bone marrow, which provides the cellular microenvironment supporting hematopoeisis. Mesenchymal stem cells were first described as bone-forming progenitors from the stromal fraction of rats by Friedenstein and Petrakova in 1966[1] and Friedenstein went on to pioneer *in vitro* culture methods for the isolation and differentiation of MSCs.[2] MSCs have subsequently been shown to differentiate into a number of mesenchymal cell types including osteoblasts, chondrocytes and adipocytes.[3]

Sources of Primary MSCs

MSCs are typically isolated from the stromal fraction of adult bone marrow. In fresh bone marrow, MSCs account for only 0.01-0.0001% of nucleated marrow cells.[4,5] Murine MSCs are classically obtained from the femurs and tibias of mice by flushing the marrow out of the bones with culture medium and transferring the resultant cell suspension in culture. Human MSCs can be similarly obtained from healthy volunteers by taking aspirates of bone marrow from the iliac crest and expanding on tissue-culture plastic.[6] Over recent years, MSC-like cells have also been identified in a number of different tissues.[2] Cells exhibiting MSC morphology and cellular characteristics have been isolated from adult peripheral blood,[7] adipose tissue,[8] skin tissue,[9] trabecular bone,[10] as well as fetal blood, liver, bone marrow[11] and lung.[12] Further MSC-like populations have been discovered in umbilical cord blood[13] and within the chorionic villi of the placenta.[14] Amniotic fluid has also been cited as a source of MSCs, with potential far-reaching implications for such areas as prenatal diagnosis and gene therapy.[15]

Isolation of Primary MSCs

It is notable that, contrary to most biological systems, human MSCs are better characterized than animal MSCs. This is probably due to the fact that MSCs are easily isolated and expanded from adult human tissue collected from healthy volunteers. Mesenchymal stem cells have nevertheless been isolated from a number of other species. Along with human MSCs, the better characterized cultures are those of rat[16] and mouse[17] origin, although therapeutic potential in large animal models has been investigated with MSCs from horse,[18] cow,[19] pig,[20] dog,[21] sheep[22] and baboon.[23] Three main approaches have been described for the isolation of MSCs and can either be used independently or combined together to obtain a more homogeneous culture. The

Exhibit A

traditional isolation method relies on the fact that MSCs selectively adhere to plastic surfaces, whereas hematopoietic cells do not and can therefore be removed through medium changes.[24] Whilst this eliminates most contaminating cells, the remaining heterogeneity of the culture progressively decreases by serial passaging and after a number of passages the culture is enriched in the self-renewing fraction, the stem cells. Another published isolation protocol involves centrifugation over a Percoll gradient, which separates cell populations based on their density and allows the enrichment of nucleated cells.[4] However, both methods are quite nonspecific and an approach that is now increasingly being used, resorts to sorting of bone marrow populations by flow cytometry (FACS), based on MSC reactivity to a number of antibodies. This can either be achieved by positively selecting for expressed antigens or by a process of immunodepletion of cells expressing hematopoietic and/or other lineage antigens. For instance, antibodies against CD34, a surface marker found on hematopoietic cells, are frequently used to identify and remove nonmesenchymal cells from a marrow culture.[3] Since there is no single specific marker available to unequivocally identify the MSC, different groups have opted for a variety of marker combinations. MSCs appear relatively stable as primary cultures[25,26] although spontaneous transformation events have been observed in long-term cultures.[27]

Characterization of MSCs

Markers used by different groups to identify the MSC fraction from human bone marrow include, but are not limited to, CD13, CD29, CD31, CD44, CD54, CD63, CD73, CD105, CD106, CD140b, CD166 and Strol.[3,28-32] Comparisons of the various combinations used by different investigators show that the majority of subsets include either CD29, CD105 or both. We have established that over 95% of cells from the human MSC culture used in our lab express both CD29 and CD105 [Figure 1B]. Although these markers have been used by various groups, there is still no general consensus on the optimal marker combination for MSCs. Some of this conjecture may be due to variations in sample origin, culture techniques and media composition among laboratories or differences in the age of the donors from which the MSCs were obtained and used for immunophenotyping. Because different antibody subsets are likely to selectively isolate slightly different cell types, comparison and evaluation of published data arising from different groups can be difficult. For instance, some groups report a degree of heterogeneity in their cultures after isolation and purification, with occasional description of a subset of small rounded cells among the more common fibroblast-like phenotype[33] [Figure 1A]. This ambiguity begs the question of what MSCs are: do they purely represent the proliferating fibroblastic-like progenitors from the bone marrow stroma or do they include all cells capable of forming mesenchymal tissue? In the absence of a specific cell marker, MSCs may well incorporate a number of different cell populations all potentially variable in their phenotypic and growth characteristics, with mesenchymal differentiation as a common denominator.

Differentiation Potential

MSCs are usually grown as a monolayer culture in medium typically containing 10% fetal calf serum at 37°C in a humid environment containing 5% CO_2. As for many other adult stem cells, MSCs are traditionally considered to only be capable of differentiating into cell types of their own original lineage, i.e., mesenchymal derivatives. We and many other groups have shown MSCs to be capable of forming osteoblasts, chrondrocytes and adipocytes both *in vitro*[34] and *in vivo*.[35] The ability of clonally expanded cells to form these three distinct cell types remains the only reliable functional criterion available to identify the genuine MSC and distinguish it from preosteoblast, preadipocyte or prechondrocytic cells which each only give rise to one cell type.[36]

Osteogenic potential

Osteogenic differentiation of MSCs is induced *in vitro* by treating a monolayer culture with a pro-osteogenic cocktail. Standardly used differentiation medium consists of dexamethasone, ascorbic acid-2-phosphate and beta-glycerophosphate.[37] Mineralized deposits can appear after a week, but the treatment is often maintained for up to three weeks in order to maximize the number and size of mineralizing nodules. Additional calcium is also used to increase *in vitro* mineralization.[10] Early signs of osteogenesis include an increase in bone-specific alkaline phosphatase activity, which can be measured enzymatically.[38] At the end of the treatment, cells can be fixed and stained with either Alizarin-Red S solution[10] [Figure 1C] or silver nitrate for von Kossa staining,[37] which highlight the calcium phosphate deposits. Quantitative measurements of mineral deposition can then be obtained by colorimetry.[10] Furthermore, osteogenic differentiation is accompanied by the expression of genes such as osterix, cbfa1, osteopontin, osteocalcin, bone sialoprotein,[39,40] which can be monitored at the RNA and protein level.

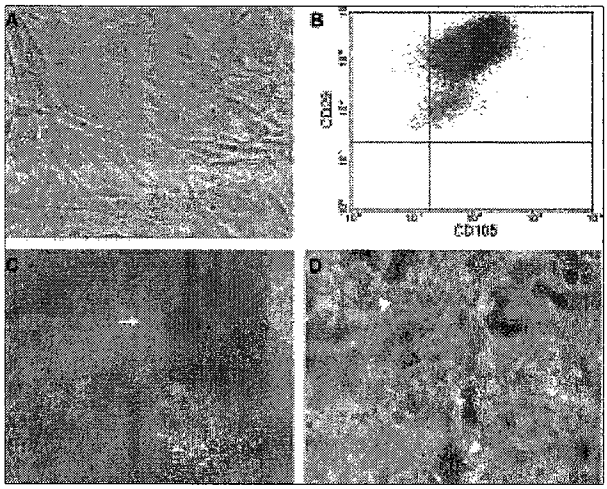

Figure 1: (A) Human MSC culture in control conditions (B) FACS analysis of control human MSCs showing co-expression of CD29 and CD105 (C) Alizarin Red S staining of human MSC culture after osteogenic differentiation highlighting mineralizing nodule formation (arrow) (D) Oil Red O staining of human MSC culture after adipogenic treatment, showing lipid droplets (arrowheads).

Chondrogenic potential

Chondrogenic differentiation is traditionally achieved by first forcing aggregation of 200,000 to 300,000 MSCs in chondrogenic medium, in order to generate a micromass pellet culture. Common to most culture protocols is the presence of dexamethasone, ascorbic acid phosphate and ITS+ supplement, which consists of bovine insulin, transferrin, selenous acid, linoleic acid and bovine serum albumin.[10] Further additives to the medium include sodium pyruvate, proline, L-glutamine and TGF-beta1,[37,41] a growth factor involved in chondrogenesis *in vivo*.[42] Over the period of treatment, there is an upregulation of chondrogenic markers such as collagen II, collagen XI, aggrecan, perlecan and syndecan.[19,43] After two to three weeks in culture, micromass pellets can then be fixed and embedded for sectioning and subsequent staining with Safranin-O, Toluidine blue or Alcian blue to highlight acid mucopolysaccharides, glycosaminoglycans and proteoglycans respectively.[10,37,41]

Adipogenic potential

Formation of mature adipocytes occurs following treatment of MSCs with a medium supplemented with dexamethasone, isobutylmethylxanthine, insulin and a PPARgamma agonist such as BRL 49653[10] with twice weekly medium changes for three weeks. Alternatively, MSCs can be exposed to three cycles of a treatment which alternates three days of culture in induction medium followed by two days in maintenance medium.[37] In this instance, the induction medium contains indomethacin instead of BRL 49653 and the maintenance medium contains insulin. The appearance of adipocytes containing lipid-filled droplets can be demonstrated by staining with oil red O (Figure 1D) and by RT-PCR detection of adipsin, aP2 and PPARgamma expression.[3,44] Quantitative data can be obtained by enzymatic dosage of the Glycerol-3-phosphate dehydrogenase, a marker of the mature adipocyte.[45] Furthermore, an elegant technique developed by Gimble allows the quantitation of adipocytes by flow cytometry in a MSC culture stained with the lipophilic dye nile red.[46]

Myogenic potential

In recent years, the extent of MSC multipotency has been frequently discussed, with many authors suggesting that MSCs have a differentiation potential broader than initially thought. When treated with the demethylating agent 5-azacytidine, MSCs have been demonstrated to have the ability to form muscle cells.[47] Myogenic potential has also been observed in a xenogeneic *in vitro* model where human MSCs are co-cultured with murine skeletal myocytes.[48]

Furthermore, MSCs have been shown to form cardiomyocytes,[49] which is being pursued as a potential replacement for the scar tissue formed as a result of myocardial infarction. Murine MSCs were reported to start beating spontaneously two weeks after 5-azacytidine treatment as well as assuming a cardiomyocyte-like morphology. The cells displayed both sinus node-like and ventricular cell-like action potentials and the beating rate was increased by isoproterenol treatment and blocked by a beta1-selective blocker, thus exhibiting signs of physiological cardiomyogenic response. This differentiation was supported by expression of alpha1, alpha1B, alpha1D, beta1 and beta2 adrenergic and M1 and M2 muscarinic receptors.[49] In all cases of MSCs displaying myogenic potential *in vivo*,[48,50] it is not clear how much of this ability is due to *de novo* myogenesis and how much is due to cell fusion events with resident myoblasts.[51] Whatever the mechanism may be, MSCs appear to provide a positive contribution to areas of muscular or cardiac damage, with no observed detrimental side-effects.[14,52]

Neural potential?

Perhaps most interesting are the recent reports that under certain conditions MSCs can be made to form neural cells. Most studies showing the formation of both neuronal and glial cells from MSCs have been carried out *in vitro*,[53,54] complemented in a few instances by *in vivo* data,[55,56] although in many cases the possibility of cell fusion cannot be formally ruled out. There have been a number of different approaches reported to trigger this apparent transdifferentiation *in vitro*. Some groups have used chemical treatments such as DMSO,[23,57] whilst others have opted for the use of growth factors such as fibroblast growth factor (FGF), neuronal growth factor (NGF) and epidermal growth factor (EGF).[53,54,58,59] However, recent evidence suggests that early positive results obtained using DMSO-based protocols are unreliable, as the neural-like morphology and gene expression displayed by MSCs after treatment were in fact due to toxicity.[60,61] Despite this setback, many other groups have reported the production of MSC-derived neurons, astrocytes,[62] oligodendrocytes[53] and Schwann cells[63] *in vitro* with varying degrees of efficiency. There is, however, an intense ongoing debate about the nature of these differentiation responses. Are they merely an artefact of the *in vitro* process or do they represent genuine neural potential from MSCs? As mentioned earlier, the multipotency of the MSC seems to be a fluid and evolving concept as novel culture conditions and differentiation treatments are being developed.

Evaluating MSC Potential for Therapeutic Applications

An ideal source of adult stem cells?

The availability of autologous MSCs, which are easily accessible from patients, makes them a promising source of cells for many clinical applications in the evolving field of regenerative medicine. As well as providing the scaffolding (stromal) fraction of the bone marrow for HSCs to proliferate on, MSCs are thought to play a role in hematopoiesis itself.[4] MSCs have been shown to significantly improve hematopoietic recovery in patients receiving high-dose chemotherapy when compared with autologous blood stem cell transfusion alone.[64] Koc *et al*[64] co-infused culture-expanded MSCs with autologous blood stem cells in breast cancer patients and observed accelerated hematopoietic recovery.

Furthermore, MSCs represent an advantageous cell type for allogenic transplantation as evidence suggests that MSCs are immune-privileged, with low MHC I and no MHC II expression,[65] therefore reducing risks of rejection and complications for transplantation. *In utero* transplantation of

human MSCs into sheep have shown that grafted cells could integrate into a variety of host tissues without any specific immune response.[66] MSCs have also been found to be immunosuppressive, through a mechanism thought to involve paracrine inhibition of T- and B-cell proliferation[67] (reviewed in[65]) and as such have been used in trials investigating their effects on autoimmune diseases and GVHD.[68,69] Co-infusion of donor-derived MSCs together with HSCs has been shown to reduce the incidence and severity of GVHD in sibling allografts.[70] It was recently reported that a nine-year-old patient suffering from progressive severe GVHD that was unresponsive to classical therapy was treated with a MSC intravenous transplant from his mother and demonstrated a complete recovery.[68] The hypo-immunogenic properties of MSCs are considered by some to be sufficient to allow transplantation even between individuals who are not HLA-compatible.[71]

Applications for osteochondral repair

MSCs represent one of the few adult stem cell populations already in use in the clinic. MSCs are currently used to develop new therapies for a number of skeletal conditions. The osteogenic potential of MSCs has been utilized to treat cases of defective fracture healing, both alone and in combination with scaffolds to repair large bone defects with a high degree of success.[72] MSCs have also been used for cartilage repair. Autologous MSCs were expanded *ex vivo*, embedded in a collagen gel and re-implanted into areas of articular cartilage defect in osteoarthritis patients.[73] In this study, formation of hyaline cartilage-like tissue was improved in the experimental group compared to control. Although most applications for tissue repair involve local transplantation of MSCs to directly target the area of injury, systemic transplantation of MSCs has been in place for a long time in hematopoietic stem cell transplants. Recently, children suffering from osteogenesis imperfecta were treated systemically with allogenic MSCs. Transplanted MSCs were shown to migrate to the bone and produce collagen, thus providing a new and efficient route to alleviate the debilitating consequences of this genetic condition.[74]

Potential applications of MSCs for myocardiac repair

Current clinical trials are investigating the potential of MSCs for the treatment of myocardial infarction.[75] As previously discussed, a number of groups have reported MSC differentiation into cardiomyocytes *in vitro*. The current *in vivo* approach consists of injecting undifferentiated MSCs or whole bone marrow directly into the heart and, although the underlying mechanisms remains to be elucidated, significant improvement has been detected.[76,77] The report by Chen *et al*[78] demonstrates a significant and sustained improvement in global left-ventricular ejection fraction, suggesting that MSC infusion triggers the formation of new cardiomyocytes and neoangiogenesis in the human heart.[79] It is still unclear whether MSCs act directly by *in situ* differentiation or fusion with resident myocytes[48] or indirectly through secretion of pro-myogenic factors promoting endogenous myocardial repair, such as VEGF and FGF.[80]

Therapeutic potential beyond mesenchymal lineages

The ability of MSCs to migrate to the site of injury has also been reported following transplantation in the brain. MSCs transplanted into rat striata were seen to migrate across the corpus callosum and populate the striatum, thalamic nuclei and substantia nigra of the 6-OHDA-lesioned hemisphere.[81] Untreated MSCs systemically infused into animals with damaged brain tissue have also been seen to migrate to the trauma site and improve recovery, although whether this is via secretion of neuroprotective factors or by differentiation into neural tissue remains unclear. Whilst it is not disputed that the MSCs appear to serve a positive role in recovery, there is debate as to whether the signs of differentiation observed *in situ* are genuine or merely a result of cell fusion with resident neural cells. More research is required to determine the reality and precise extent of MSC contribution in brain repair models as such results could have implications for neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease or traumatic events such as stroke or spinal cord injury. Although the adult brain contains populations of neural stem cells[82,83] these are insufficient to replace the massive number of cells needed to treat these conditions. The critical question has been to identify a source of neural progenitor cells for transplantation. The potential use of fetal tissue or differentiated embryonic stem cells suffers limitations due to tissue availability, ethical issues or safety concerns. The identification of an adult population of cells such as MSCs, which can be easily obtained from autologous or donated marrow and can be cultured and manipulated *ex vivo*, would represent a significant breakthrough in the search for many applications in regenerative medicine.

Conclusion

Mesenchymal stem cells represent a population of cells with the potential to contribute to future treatments for a wide range of acute or degenerative diseases. Significant progress has been made to identify the pharmacological and molecular pathways driving MSC differentiation towards mesenchymal derivatives *in vitro* and preliminary results indicate that MSCs could be used to generate neural derivatives. Much remains to be done in order to evaluate the physiological relevance of these early observations and to unravel the molecular mechanisms governing their differentiation *in vivo*. Applications currently under investigation for MSC-based therapies include musculoskeletal and cardiac repair, as well as genetic manipulation of MSCs for gene therapy strategies. Directed differentiation of autologous MSCs towards extra-mesenchymal lineages is an exciting and promising area of stem cell biology, with potential for the repair of tissues where resident stem cells are not accessible, such as the brain. MSCs thus represent an interesting and versatile population of adult stem cells demanding further molecular characterization and functional investigation. Future research will define the extent of their potential as an autologous and allogenic stem cell source for clinical application.

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